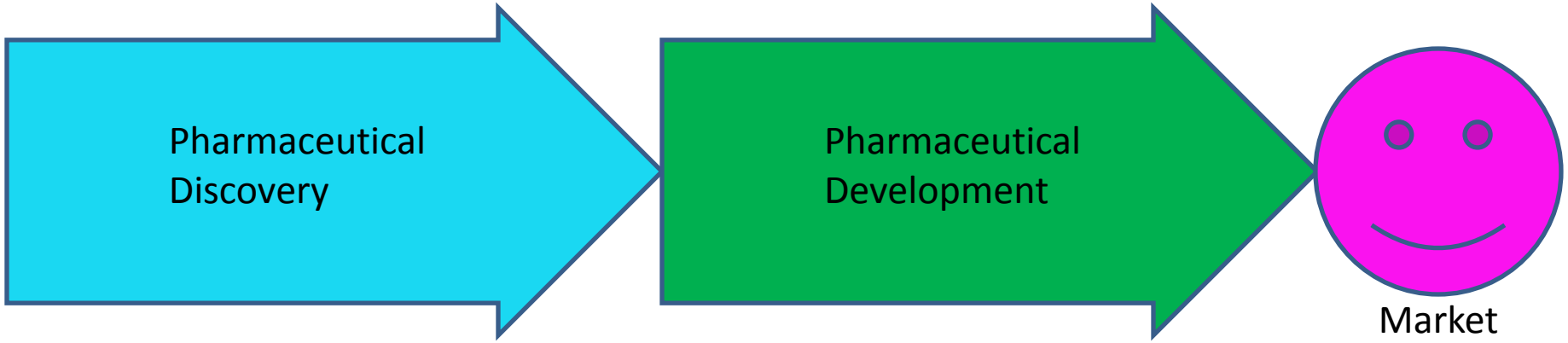
Pharmaceutical Macromolecular Crystallography at Synchrotrons

Steven Sheriff

Bristol-Myers Squibb R&D

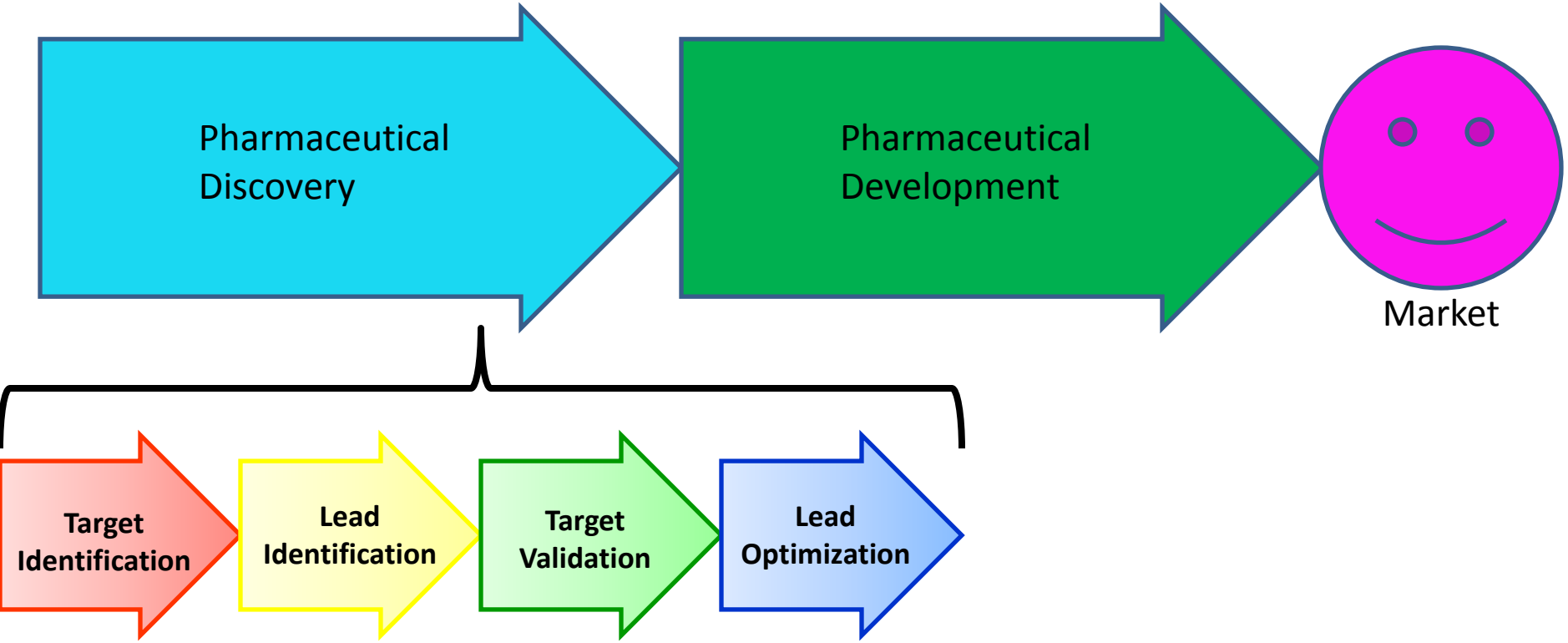
Drug Discovery & Development Process



Goal: Discover molecule with appropriate drug properties

Goal: Demonstrate safety and efficacy of drug candidate in humans

Drug Discovery & Development Process



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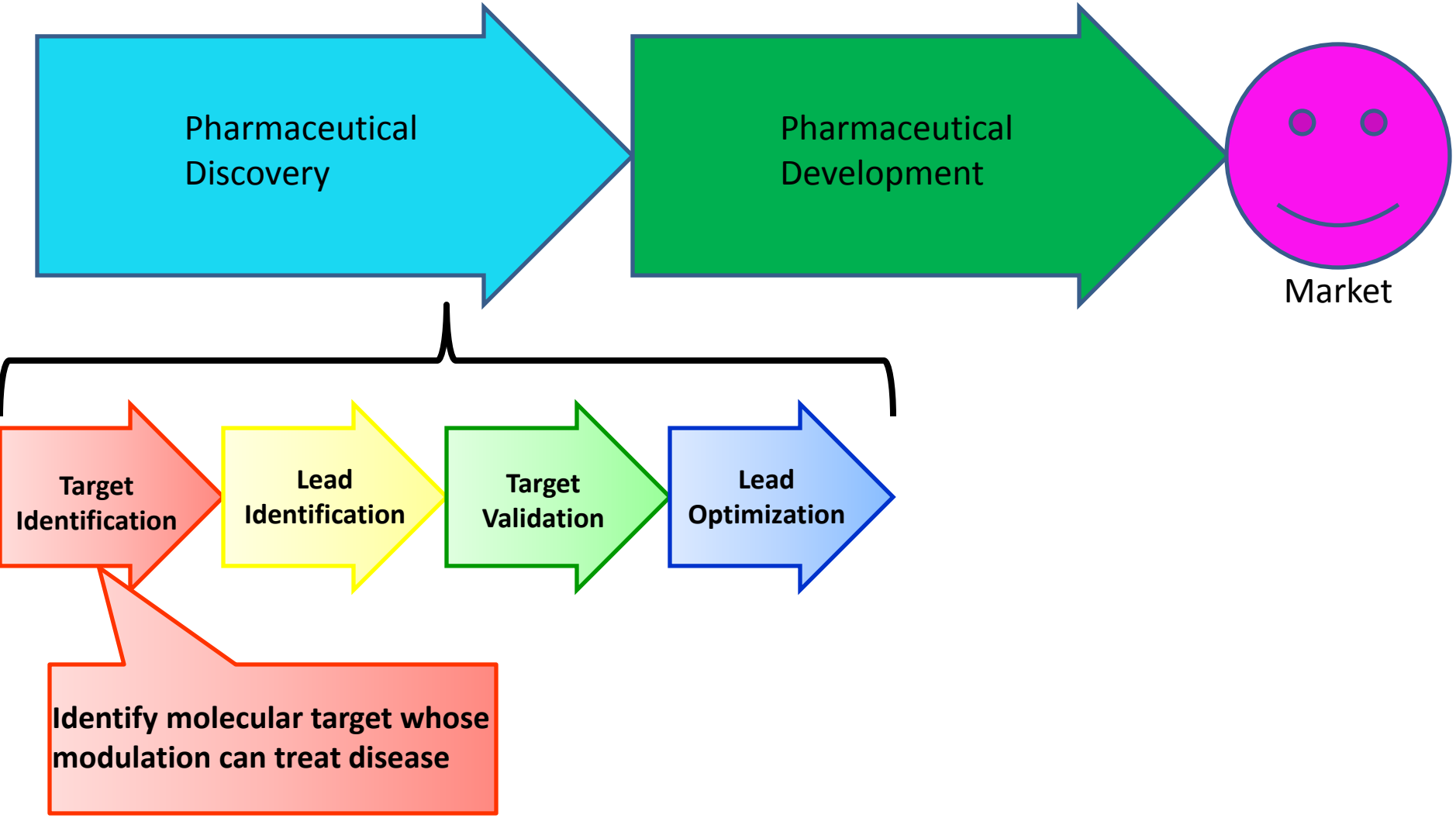

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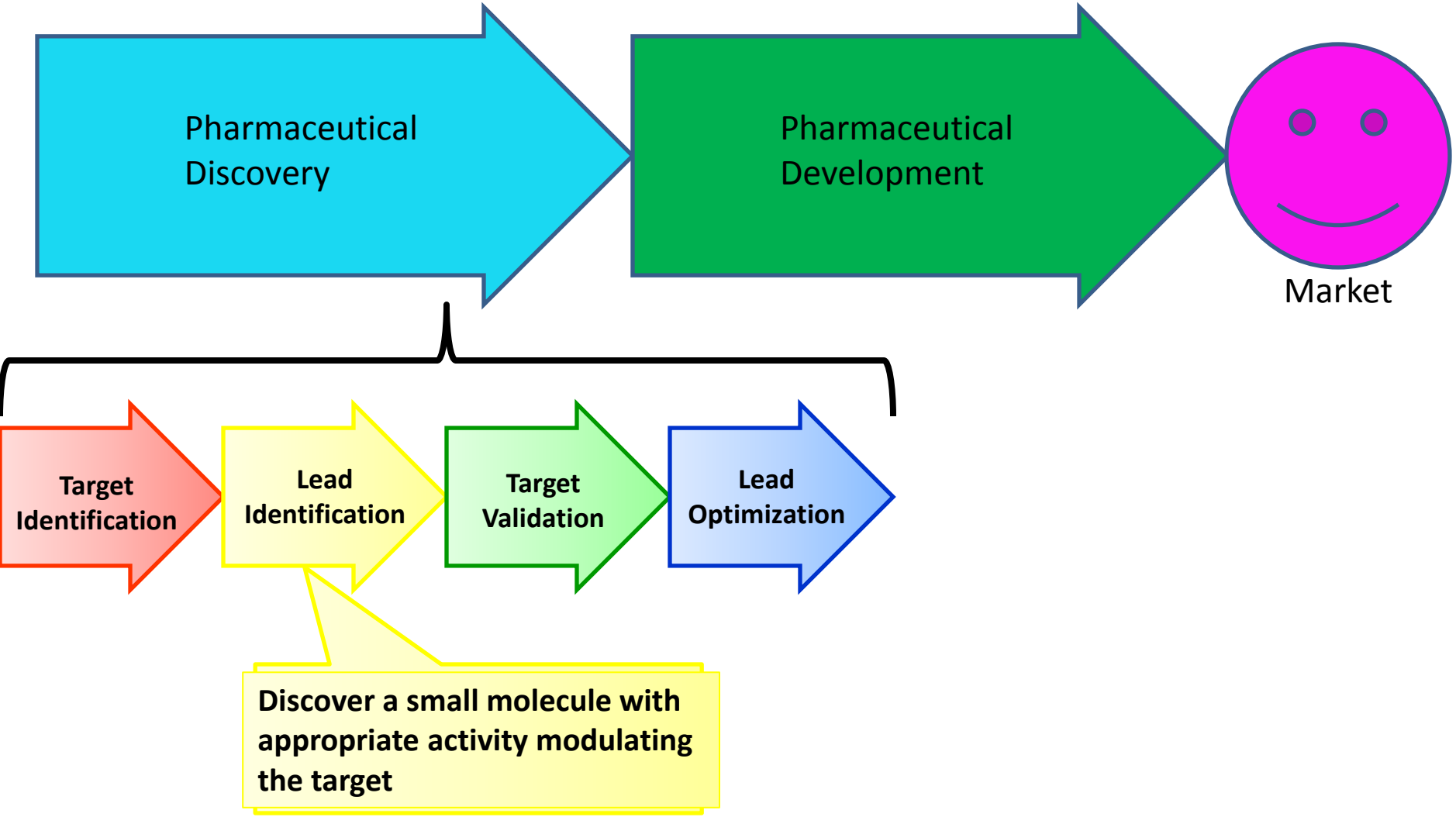

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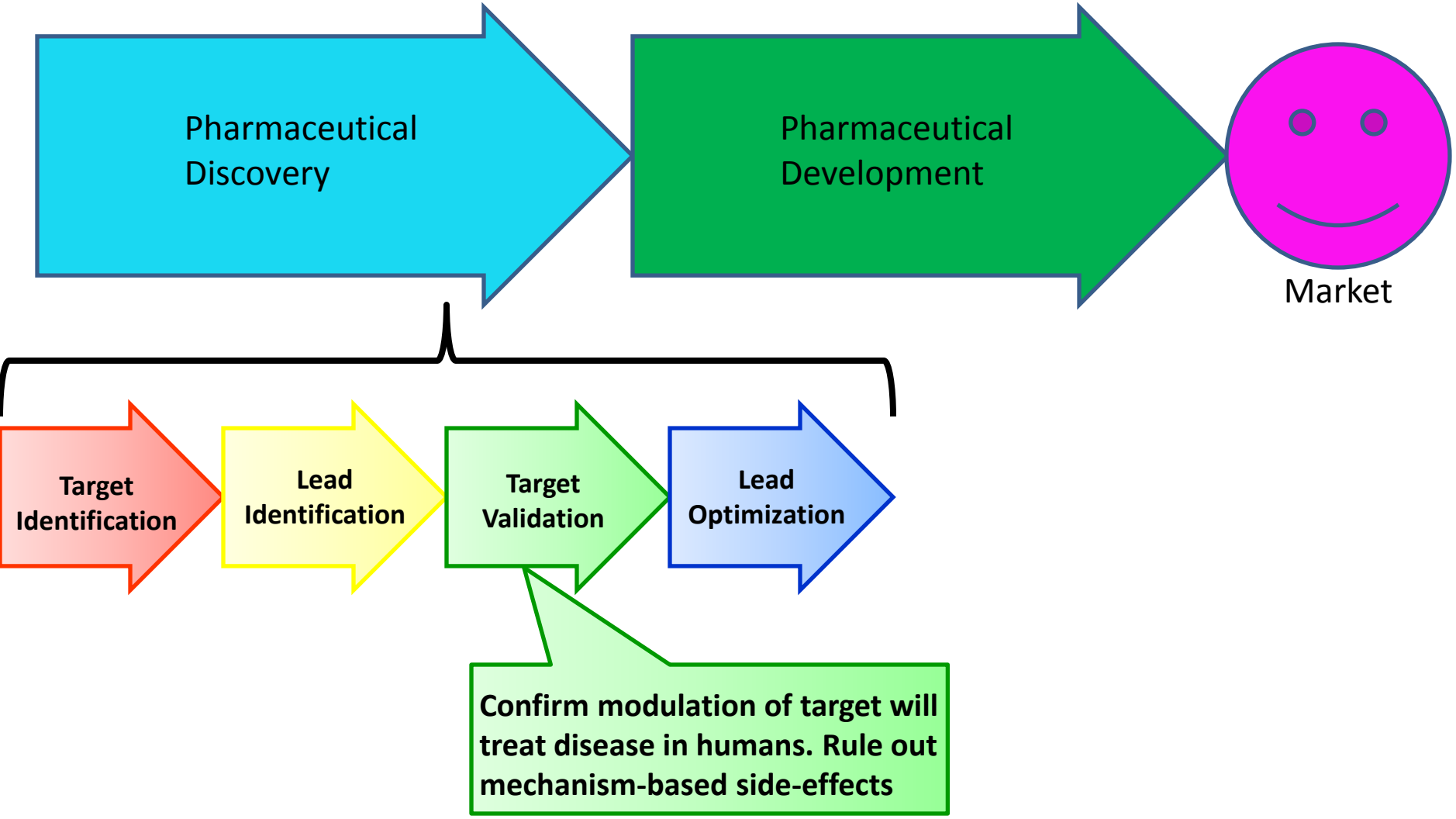

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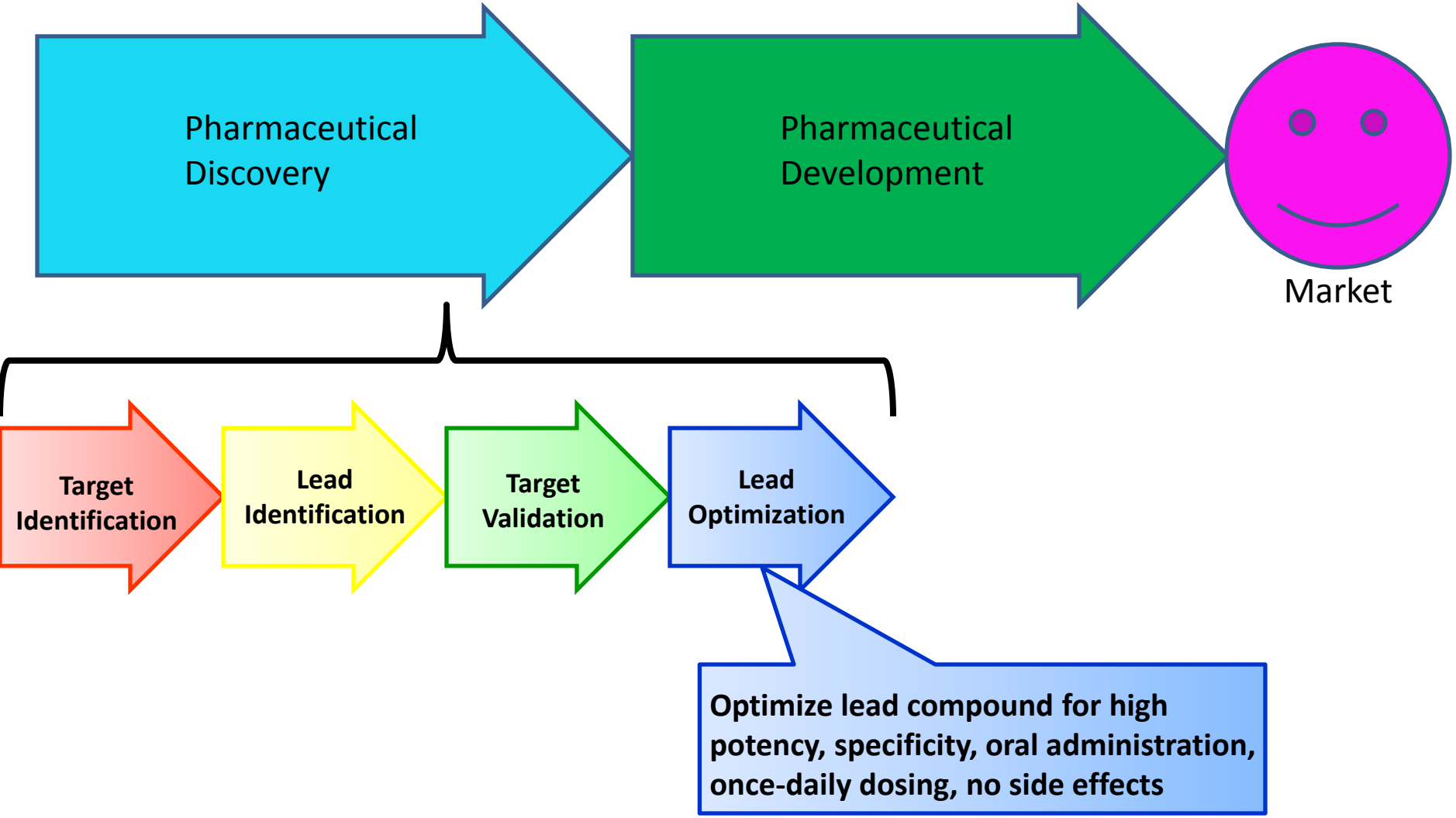
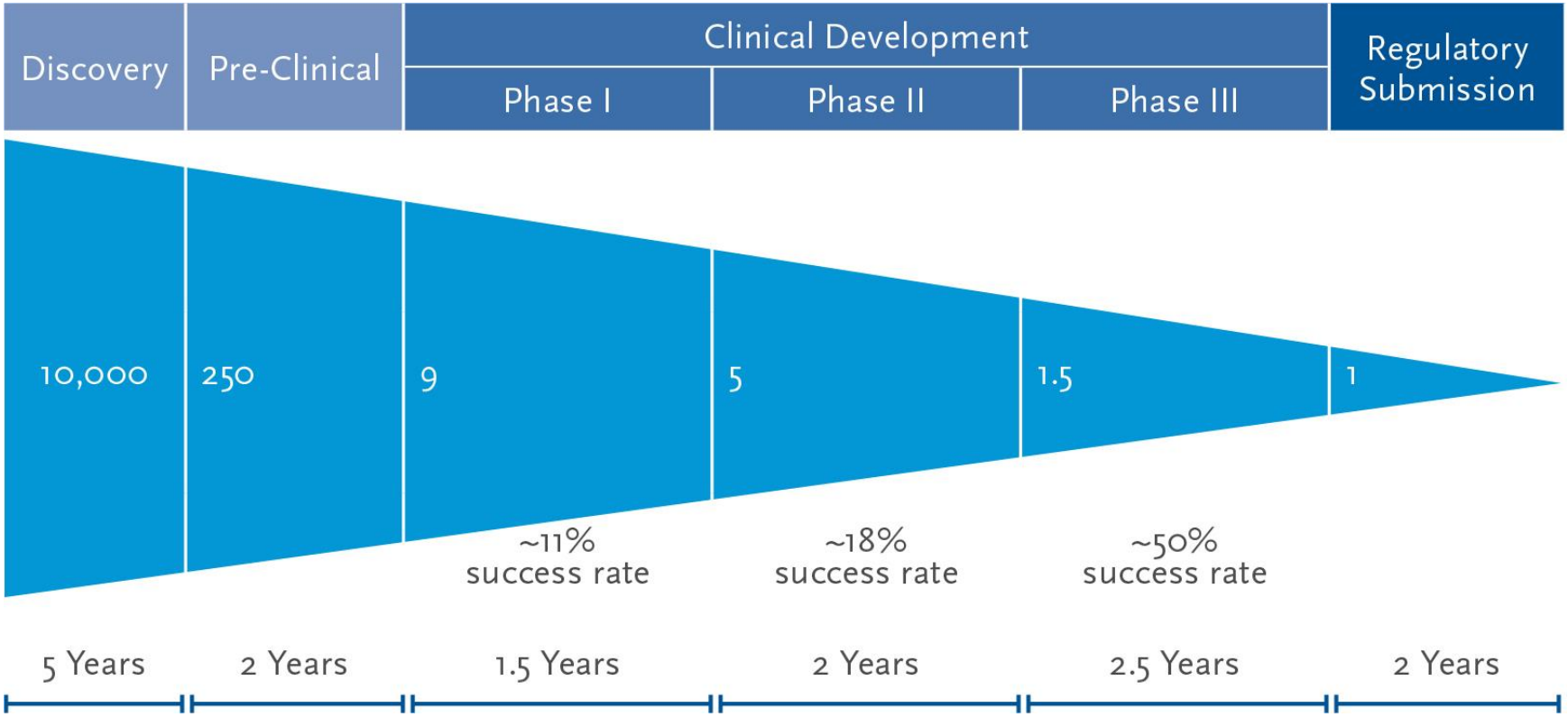


Drug Discovery Process – Attrition



- From "Strategic Portfolio Management & New Influencers in R&D Decision-Making" available at: https://www.quintiles.com/assets/0/111/118/233/1336/313de5b8-3332-4bf5-a654-f2396c58e231.pdf

Small Molecules

- Traditional pharmaceutical macromolecular crystallography
  - Part of an iterative structure-based drug design process
  - Depending on the crystallographic difficulty of the target number of structures might range from 1-2 to >100.
  - This rubric would also include fragments, examination of "top" high-throughput-screening hits, and molecules smaller than small proteins, but larger than traditional small molecule drugs.
  - Might also include work on anti-targets, proteins with similar enzymatic or binding specificity, or off-targets, e.g. proteins involved in xenobiotic degradation

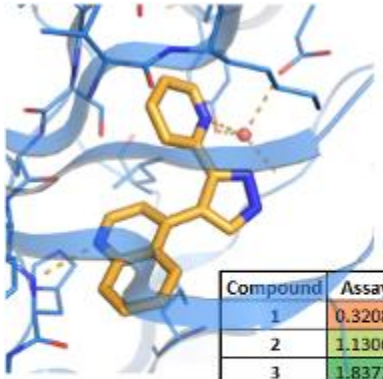
Structure-based Drug Design Cycle

Starting points:
- HTS hits
- Fragments
- Virtual screen hits

Chemical Ideas

Chemical Ideas

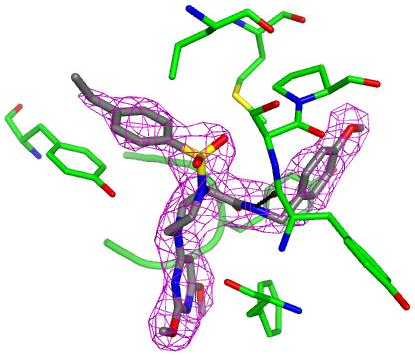
Molecules



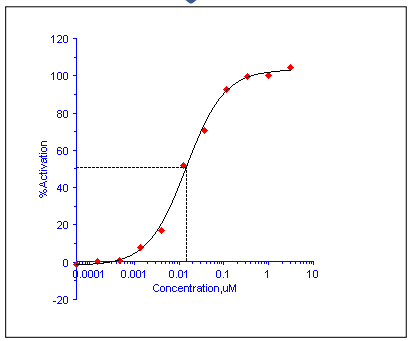
Compound	Assay 1	Assay 2	Assay 3
1	0.320808	0.061107	1.583531
2	1.130641	0.967821	0.685535
3	1.837198	1.232571	0.106721
4	0.207122	1.107845	0.462774
5	1.671795	0.663326	0.136387
6	0.578837	0.61373	1.655686
7	0.099022	0.047063	0.30917
8	1.806055	1.919455	0.831756
9	0.029873	1.515186	0.442097
10	0.029594	0.552144	0.635703

CADD

Chemical Ideas



Structure Determination



Assays

Small Molecules Continued: Fragments

- Fragment-based approaches have big impacts on pharmaceutical crystallography synchrotron usage
  - Lots of data sets need to be collected.
  - Compared to medicinal chemistry generated compounds relatively few data sets will have compound bound, even when the compound is first screened by biophysical techniques, e.g. NMR, Surface Plasmon Resonance, or Thermal Shift Analysis.
    - Fragments typically don’t have sufficient potency to use biochemical assays

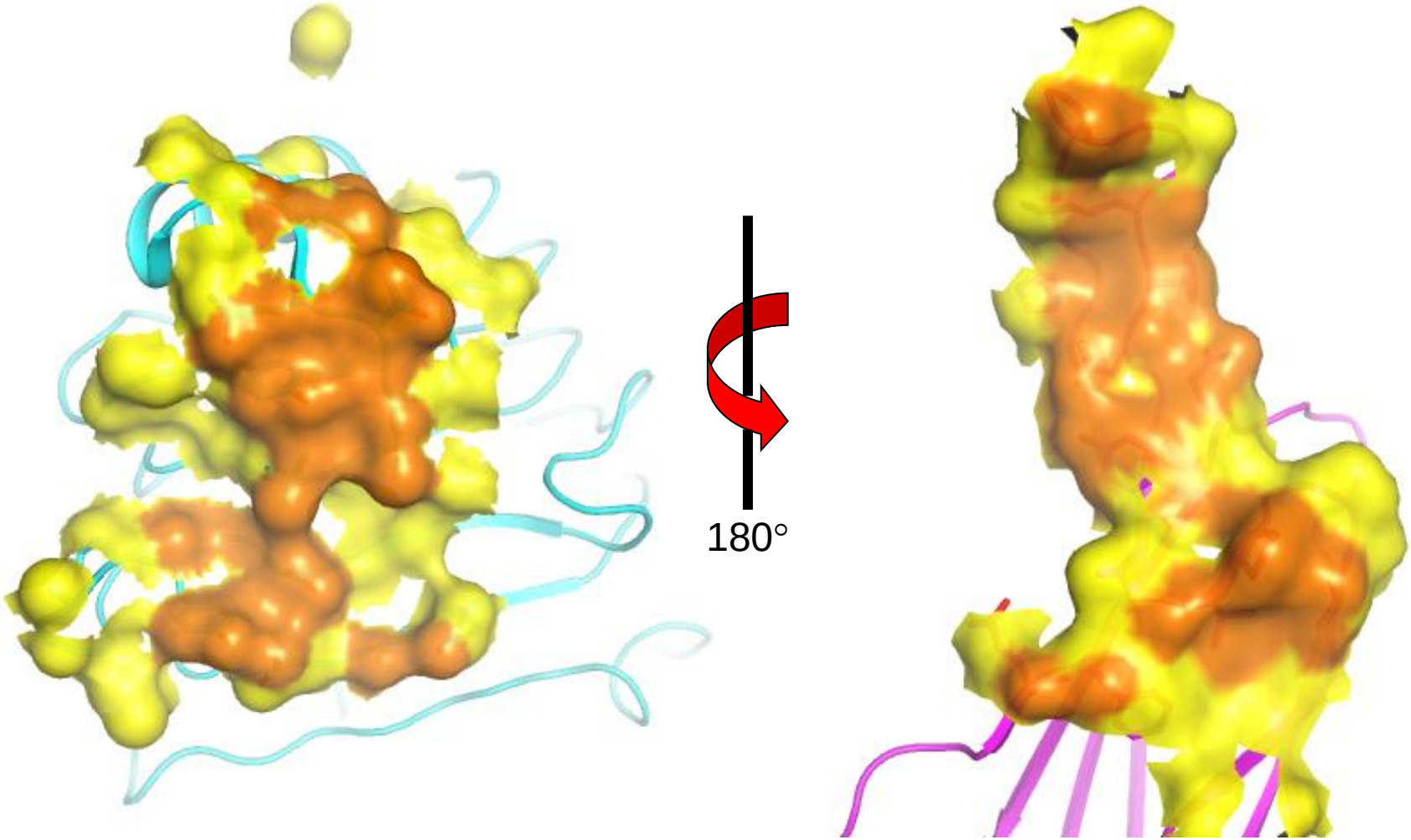
Fragments Continued

- High resolution (better than 2 Å) is essential as compound may only partially occupy site and/or may be highly mobile.

Biological Macromolecules

- These are antibodies or other proteins that attempt to mimic antibodies or native proteins such as erythropoietin
- Principal aim for these structures is to map the epitope, which is the binding surface on the target protein, and the paratope, which is the binding surface on the “drug” molecule.
  - Typically, one or at most a handful of structures.
  - Often used only for IP, but increasingly used earlier in the process to help optimize the protein

Epitope and Paratope Mapping



Epitope

Buried
In contact

Paratope

BMS Synchrotron Use

- While APS is running, we have weekly access at IMCA (17-ID) of alternately 10 or 14 hour slots (30+ weeks/year).
  - We contract with Shamrock Structures to collect data in preference to collecting data remotely.
    - If necessary, they can remove frost from crystals.
    - Fix or call in beamline help when mechanical or computer problems occur.
    - If the beam dumps, they can stop and restart.

BMS Synchrotron Use Continued

- Process data. Shortly after the end of a run, we have all data and log files available via their secure server.
- Raw data is returned on a USB drive with samples.

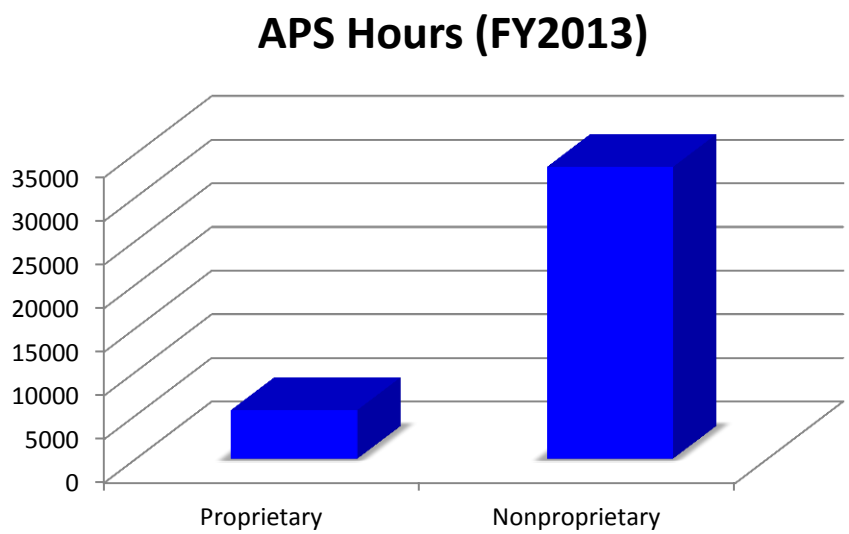
– Between 6 and 10 data sets can be collected per hour
– With Pilatus 6M detector much more time is spent exchanging samples and centering (~4 minutes) than in data collection (~2 minutes).
– Typically use >90% attenuation at IMCA.

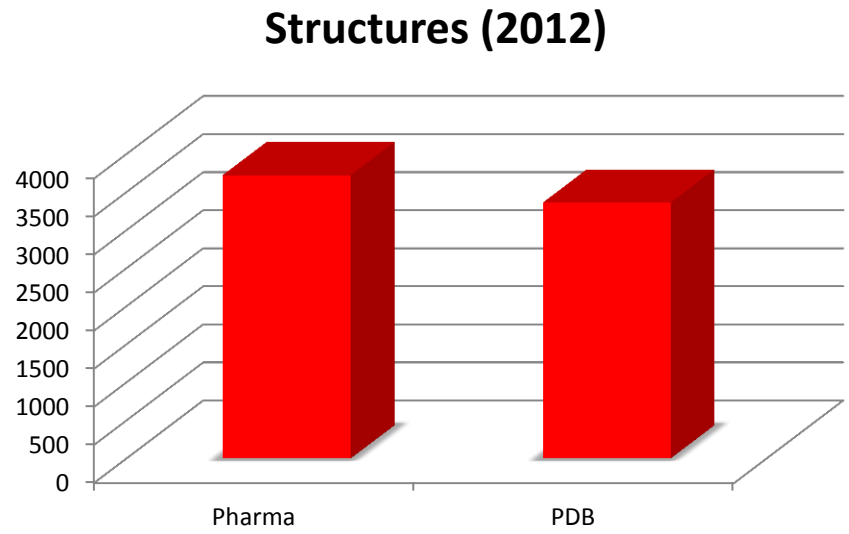
BMS Synchrotron Use Continued

- Unlike academic crystallographers we rarely collect data to be used for experimental phasing, e.g. SAD or MAD.
  - However, we do determine structures of proteins that are not available in the literature, but molecular replacement typically suffices.

- When APS is not running, we contract with Shamrock for approximately biweekly access at CLS 08ID-1.

Productivity at US Light Sources





- Hours for Macromolecular Crystallography (MX) at the APS
- Structures from survey of US pharmaceutical research (10 companies)

From Stephen Wasserman's 2014 AAAS NUFO session talk, who, in turn, acknowledges IMCA, Amgen, Genentech, Glaxo SmithKline (US), Lilly, and the APS User Office for data. S.W. notes that two large US-based pharmaceutical companies did not participate suggesting that number of structures from pharmaceutical companies would be even larger.

Recommendations – All Macromolecular Crystallographers

- US Synchrotron sources should coordinate their scheduled downtimes so that at least one US synchrotron is running at “all” times.
  - An approximately two-week hiatus at the end/beginning of a calendar year would be acceptable.
- Since macromolecular crystallography experiments typically take <<1 day, a rapid access program with minimal paperwork.

Recommendations – Industrial Macromolecular Crystallography

- Develop one or more mechanisms whereby a fee-for-service or in-kind model allows facility staff to be able to collect data for companies without raising IP ownership concerns.
  - Also potentially useful for occasional use application, e.g. SAXS at the NIH-funded APS 18-ID

Acknowledgements

- My colleagues at BMS
- For figures
  - Andy Tebben
  - Liang Schweizer
- Steve Wasserman, Lilly CAT for his AAAS 2014 NUFO session slide deck
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